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VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that:

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That I am knowledgeable in the English language and in the language in which the below identified Japanese application was filed, and that I believe that the English translation of the Japanese patent application No. 2000-094333 filed on March 30, 2000 attached hereto is a true and complete translation of the above-identified Japanese application as filed.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Ion Selective Electrode

5 [Claims]

[Claim 1] A composite ion selective electrode comprising a calcium ion selective electrode member and a hydrogen ion selective electrode member, each of which comprises an electro-insulating support, a pair of electrode units each of which comprises a silver layer and a silver halide layer electrically separated from each other, an electrolyte layer, and an ion selective membrane; an electro-insulating member having one opening for introducing a sample liquid and another opening for introducing a reference liquid; a distributing member for distributing the introduced sample liquid to the ion selective membrane; a distributing member for distributing the introduced reference liquid to the ion selective membrane; and a bridge member which is provided so as to electrically connect the sample liquid and the reference liquid;

which is characterized in that the ion selective membrane of the calcium ion selective electrode member has a thickness of 5 to 30 μm , and the ion selective membrane of the hydrogen ion selective electrode member contains tri-n-dodecylamine and trisethylhexyl trimellitate.

[Claim 2] The composite ion selective electrode of claim 1, wherein the calcium ion selective membrane of the calcium ion selective electrode member contains calcium di[4-(1,1,1,3-tetramethylbutyl)phenyl] phosphate, dioctylphenyl phosphonate and vinyl chloride-vinyl acetate copolymer.

[Claim 3] The composite ion selective electrode of claim 1 or 2, wherein the thickness of the ion selective membrane of the calcium ion selective electrode member is less than 20 μm .

[Claim 4] The composite ion selective electrode of claim 3, wherein the thickness of the ion selective membrane of the calcium ion selective electrode member is in the range of 10 to 18 μm .

5 [Claim 5] The composite ion selective electrode of any claim of 1 to 4, wherein the thickness of the ion selective membrane of the hydrogen ion selective electrode member is in the range of 5 to 30 μm .

10 [Claim 6] The composite ion selective electrode of any claim of 1 to 5, wherein the electrolyte layer of each ion selective electrode member comprises sodium chloride.

[Claim 7] A method for determining a standardized concentration of a calcium ion at pH 7.4 in a sample blood employing the composite ion selective electrode of any claim of 1 to 6, which comprises the steps of measuring a calcium ion concentration and a hydrogen ion concentration in a sample blood and incorporating the measured calcium ion concentration and the measured hydrogen ion concentration into the following equation:

$$\text{Log } (i\text{Ca at } 7.4) =$$

$$\text{Log } (i\text{Ca at pH}) - 0.22 \times (7.4 - \text{pH})$$

in which $i\text{Ca}$ means a calcium ion concentration and pH means a hydrogen ion concentration.

25 [Claim 8] An ion selective electrode for analysis of a calcium ion comprising an electro-insulating support, a pair of electrode units which comprise a silver layer and a silver halide layer electrically separated from each other, an electrolyte layer, and a calcium ion selective membrane, which are laminated in this order, an electro-insulating member having an opening for introducing a sample liquid into the above one electrode unit, an electro-insulating member having an opening for introducing a reference liquid into the other electrode unit, and a bridge member for electrically connecting the introduced sample liquid and the introduced reference

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liquid at the opening, wherein the calcium ion selective membrane has a thickness of 5 to 30 μm .

[Claim 9] The ion selective electrode of claim 8, wherein the ion selective membrane contains calcium
5 di[4-(1,1,1,3-tetramethylbutyl)phenyl] phosphate, dioctylphenylphosphonate and a vinyl chloride-vinyl acetate copolymer.

[Claim 10] The ion selective electrode of claim 8 or 9, wherein the thickness of the ion selective mem-
10 brane is less than 20 μm .

[Claim 11] The ion selective electrode of claim 10, wherein the thickness of the ion selective membrane is in the range of 10 to 18 μm .

[Claim 12] The ion selective electrode of any
15 claim of 8 to 11, wherein the electrolyte layer comprises sodium chloride.

[Claim 13] An ion selective electrode for analysis of a hydrogen ion comprising an electro-insulating support, a pair of electrode units which comprise a silver layer and a silver halide layer electrically separated from each other, an electrolyte layer, and an ion selective membrane, which are laminated in this order, an
20 electro-insulating member having an opening for introducing a sample liquid into the above one electrode unit, an electro-insulating member having an opening for introducing a reference liquid into the other electrode unit, and a bridge member for electrically connecting the introduced sample liquid and the introduced reference liquid
25 at the opening, wherein the hydrogen ion selective membrane contains tri-n-dodecylamine and trisethylhexyl trimellitate.
30

[Claim 14] The ion selective electrode of claim 13, wherein the ion selective membrane comprises tri-n-dodecylamine, trisethylhexyl trimellitate, potassium-
35 tetrakis(p-chlorophenylborate) and a vinyl chloride-vinyl acetate copolymer.

[Claim 15] The ion selective electrode of claim 13 or 14, wherein the ion selective membrane has a thickness of 5 to 30 μm .

5 [Claim 16] The ion selective electrode of any claim of 13 to 15, wherein the electrolyte layer comprises sodium chloride.

[Detailed Description of Invention]

[Fields of Invention]

10 This invention relates to an ion selective electrode of wet type employable for determining a concentration of a calcium ion and/or a hydrogen ion. In particular, the invention relates to an ion selective electrode for potentiometrically determining a concentration of a calcium
15 ion and/or a hydrogen ion existing in body fluids such as blood and serum, and to a process for determining the ion concentration employing the ion selective electrode.

[Prior Art]

20 Methods for detection of the concentration of a specific ion existing in liquids (e.g. city water, river water, sewage and industrially waste water) and body fluids (e.g. blood, urine and saliva) employing ion selective electrodes have been already known.

25 Wet method and dry method are known. In the wet method, a barrel type electrode having a standardized liquid in the electrode is employed. This method has defects that the handling is troublesome from view points of maintenance, washing, conditioning, longevity and breakage and that a large amount of sample liquid more
30 than several hundreds μL is required for immersing a needle-shaped electrode in a sample liquid.

35 In order to remove above disadvantages, a method employing dry type ion selective electrode in the form of film has been proposed. A dry type ion selective electrode is a device (ion concentration determining device) for potentiometrically determining the concentration of a

specific ion contained in body fluids such as blood, urine and saliva employing an appropriate amount of body fluids. The typical structure thereof is disclosed in, for example, Japanese Patent Publications No. H3-54788 and H4-50530. The ion selective electrode has a basic structure comprising a support, an electrically conductive metal layer (e.g. a silver layer), a layer containing water-insoluble salt of the metal (e.g., silver chloride layer), an electrolyte layer containing an electrolyte salt (e.g., potassium chloride and sodium chloride) comprising an anion the same as that of said water-insoluble salt and cation (e.g., potassium ion and sodium ion) and a binder, and ion selective membrane, which are laminated in this order. The concentration of a specific ion in a sample liquid can be determined by connecting the pair of the electrode membranes to a potentiometer by means of a bridge member, spotting a sample liquid and a standardized liquid (a reference liquid) on each electrode membrane and measuring the potential difference. According to the dry method employing an ion selective electrode in the form of film, maintenance of the electrode is not required and the device has a small size. Therefore, it is possible to perform the determination of the ion concentration at a bed side and to decrease an amount of the sample liquid required for determination.

The dry ion selective electrode can determine concentration of inorganic ions such as hydrogen ion (H^+), lithium ion (Li^+), sodium ion (Na^+), potassium ion (K^+), magnesium ion (Mg^{2+}), calcium ion (Ca^{2+}), chlorine ion (Cl^-), hydrogencarbonate ion (HCO_3^-) and carbonate ion (CO_3^{2-}) by changing ion selective membranes.

A composite ion selective electrode which comprises a plurality of ion selective electrode members in one device and can simultaneously determine concentrations of two or more different ions by spotting a sample liquid and reference liquid one time has been known. Such elec-

trode is disclosed in, for example, Japanese Patent Publications No. H4-76577 and No. H5-56819.

Determination of concentration of the above inorganic ions in a body fluid is important for clinical medicine. Among them, the determination of an ionic calcium (iCa) is necessary for diagnoses of abnormal metabolism (e.g., functional disorder of accessory thyroid or transfer of cancer to bone), a monitor for supplying calcium in blood transfusion during operation (calcium ion in blood is sometimes decreased by combining with citric acid added as anti-coagulating agent), and screening of clinical tests. Also important is the determination of concentration of hydrogen ion (in term of pH) in body fluid, because the pH value teaches an acid-base balance in body fluid. In most of the clinical tests, the ionic calcium concentration in blood is generally utilized after it is standardized in terms of a concentration value at pH 7.4. Therefore, it is of value that both of the calcium ion concentration and the hydrogen ion concentration are simultaneously determined.

Since the dry ion selective electrode has a simple structure in the form of small chip as described above, it requires a very small amount of sample liquid. Therefore, it is very advantageous in case that the amount of sample liquid such as body fluid is limited. The ion selective electrode having a simple and small structure can be employed separately from a potentiometer. The electrode can be changed at each measurement. However, the ion selective electrode having small structure is apt to give insufficient accuracy in measurement. Even if ion selective electrodes are manufactured in the same manner, each electrode gives different value. Therefore, reproducibility of obtained data is not high. Further, a response period for obtaining a constant analytical data is prolonged. It is also noted that the ability of electrodes is lowered at a lapse of time after the electrodes

are manufactured. Accordingly, errors are caused in each ion selective electrode, thereby lowering reproducibility of obtained data or failing to show accurate potential.

[Problems To Be Solved by Invention]

5 It is an object of the invention to provide a composite ion selective electrode for analysis of both of a calcium ion and hydrogen ion which gives satisfactory accuracy and analytical reproducibility.

10 Further, it is an object of the invention to provide a calcium ion selective electrode which shows good accuracy and analytical reproducibility within a short response time.

15 Furthermore, it is an object of the invention to provide a hydrogen ion selective electrode which shows good accuracy and analytical reproducibility at lapse of time.

Moreover, it is an object of the invention to provide a process for accurate determination of concentration of a calcium ion in blood.

20 [Means for Solving Problems]

As a result of study of the present inventors regarding dry ion selective electrode for analysis of calcium ion and hydrogen ion, it has been discovered that the calcium ion selective electrode having a calcium ion
25 selective membrane thinner than a certain level gives good analytical results in the reproducibility of the analytical data and the response time. It is advantageous to make the ion selective electrode membrane thin to keep balance of a pair of electrodes, which is a problem peculiar to the differential potentiometry. Further,
30 it has been discovered that the calcium ion selective membrane containing calcium di[4-(1,1,1,3-tetramethylbutyl)phenyl]phosphate, dioctylphenyl phosphonate and vinyl chloride-vinyl acetate copolymer can give the analytical data of calcium ion concentration with increased
35 accuracy.

It is also discovered that the hydrogen ion selective electrode having a hydrogen ion selective membrane containing tri-n-dodecylamine and trisethylhexyl trimellitate prevents deterioration of the electrode at
5 lapse of time, thereby giving analytical data with increased reproducibility. The hydrogen ion selective membrane preferably comprises a combination of tri-n-dodecylamine, trisethylhexyl trimellitate, potassium-tetrakis(p-chlorophenyl borate), and a vinyl chloride-
10 vinyl acetate copolymer, so as to remarkably increase analytical reproducibility.

Accordingly, the present invention resides in a composite ion selective electrode comprising a calcium ion selective electrode member and a hydrogen ion selective
15 electrode member, each of which comprises an electro-insulating support, a pair of electrode units each of which comprises a silver layer and a silver halide layer electrically separated from each other, an electrolyte layer, and an ion selective membrane, which are laminated
20 in this order; an electro-insulating member having one opening for introducing a sample liquid and another opening for introducing a reference liquid; a distributing member for distributing the introduced sample liquid to the ion selective membrane; a distributing member for
25 distributing the introduced reference liquid to the ion selective membrane; and a bridge member which is provided so as to electrically connect the sample liquid and the reference liquid;

which is characterized in that the ion selective
30 membrane of the calcium ion selective electrode member has a thickness of 5 to 30 μm , and the ion selective membrane of the hydrogen ion selective electrode member contains tri-n-dodecylamine, trisethylhexyl trimellitate, potassium-tetrakis(p-chlorophenyl borate), and a vinyl
35 chloride-vinyl acetate copolymer.

The invention further resides in an ion selective

electrode for analysis of a calcium ion comprising an electro-insulating support, a pair of electrode units which comprise a silver layer and a silver halide layer electrically separated from each other, an electrolyte layer, and a calcium ion selective membrane, which are laminated in this order, an electro-insulating member having an opening for introducing a sample liquid into the above one electrode unit, an electro-insulating member having an opening for introducing a reference liquid into the other electrode unit, and a bridge member for electrically connecting the introduced sample liquid and the introduced reference liquid at the opening, wherein the calcium ion selective membrane has a thickness of 5 to 30 μm .

The invention furthermore resides in an ion selective electrode for analysis of a hydrogen ion comprising an electro-insulating support, a pair of electrode units which comprise a silver layer and a silver halide layer electrically separated from each other, an electrolyte layer, and a hydrogen ion selective membrane, which are laminated in this order, an electro-insulating member having an opening for introducing a sample liquid into the above one electrode unit, an electro-insulating member having an opening for introducing a reference liquid into the other electrode unit, and a bridge member for electrically connecting the introduced sample liquid and the introduced reference liquid at the opening, wherein the hydrogen ion selective membrane contains tri-n-dodecylamine and trisethylhexyl trimellitate.

The present invention furthermore resides in a method for determining standardized concentration of a calcium ion at pH 7.4 in a sample blood employing the above composite ion selective electrode, which comprises the steps of measuring a calcium ion concentration and a hydrogen ion concentration in a sample blood and incorporating the measured calcium ion concentration and the

measured hydrogen ion concentration into the following equation:

$$\text{Log (iCa at 7.4) =}$$

$$\text{Log (iCa at pH) - 0.22 x (7.4 - pH)}$$

5 in which iCa means a calcium ion concentration and pH means a hydrogen ion concentration.

[Preferred Embodiment of Invention]

The (composite) ion selective electrode of the invention can have various structures and a representative
10 structure of the electrode will be described with reference to the drawings.

Fig. 1 is a rough perspective view of a structure of a representative ion selective electrode for determining a calcium ion or a hydrogen ion. The ion selective electrode member of Fig. 1 is composed of an electro-insulating support 11, a silver layer 12a, 12b (the silver layer is divided into two regions by a groove which reaches to the surface of the support), a silver halide layer 13a, 13b, an electrolyte layer 14, and an ion selective membrane 15. A water-impermeable electro-insulating member
15 20 16 is provided on the ion selective membrane 15. An opening 17a for introducing a sample liquid and an opening 17b for introducing a reference liquid are provided at a position corresponding to the pair of silver layer and silver halide layer (electrode unit). A bridge member 18 is provided for electrically connecting the sample liquid and reference liquid of the openings 17a and 17b.
25

Fig. 2 is a broken perspective view of a structure of a composite ion selective electrode of the present invention. In the composite ion selective electrode of Fig. 2, ion selective electrode members 21a, 21b and 21c are arranged between an upper frame 22 and a lower frame 23. An opening 24a for introducing a sample liquid and an opening 24b for introducing a reference liquid are provided on the upper frame 22. The openings 24a and 24b
30 35 are provided with a bridge member 25 for electrically

connecting the sample liquid and reference liquid. A pair of distributing members 26a and 26b for horizontally distributing the sample liquid and reference liquid to each ion selective electrode member respectively are provided on the upper surface of the lower frame 23. An opening 27 for exposing the connecting region of each ion selective electrode member downward and an opening 28 for letting air out are provided on the lower frame 23. Three pairs of ion selective electrode members 21a, 21b and 21c have the same structure as shown in Fig. 1 and are so placed as to have ion selective membranes faced downward.

In Fig. 2, 21a and 21b are a pair of calcium ion selective electrode members and a pair of hydrogen ion selective electrode members, respectively. 21c is a pair of dummy electrodes made of a silver metal and serve for facilitating electric discharge. The composite ion selective electrode of the invention is not limited to the above three pairs of electrode members and can have pairs of other ion selective electrode members and dummy electrodes for facilitating electric discharge, as long as two pairs of ion selective electrode members are a calcium ion selective electrode member and hydrogen ion selective electrode member.

The calcium ion selective membrane of the calcium ion selective electrode member of the invention should have a thickness of 5 to 30 μm , preferably 5 to 20 μm , more preferably 10 to 18 μm in order to increase accuracy and reproducibility of the data.

The calcium ion selective membrane of the calcium ion selective electrode member of the invention can be prepared by employing known components. Preferred components are calcium di[4-(1,1,1,3-tetramethylbutyl)phenyl]phosphate, dioctylphenyl phosphonate (plasticizer) and a vinyl chloride-vinyl acetate copolymer. These components can be employed in combination with other known compo-

nents.

The hydrogen ion selective membrane of the hydrogen ion selective electrode member of the invention contains tri-n-dodecylamine and trisethylhexyl trimellitate (plasticizer) so as to increase accuracy and reproducibility of analytical data. Preferred components are tri-n-dodecylamine, trisethylhexyl trimellitate, potassium tetrakis(p-chlorophenylborate) and a vinyl chloride-vinyl acetate copolymer. These components can be employed in combination with other known components.

The hydrogen ion selective membrane has a thickness of 5 to 30 μm , preferably 5 to 20 μm to increase reproducibility of the data and to keep balance of the pair of the electrode units, which is a problem peculiar to the differential potentiometry.

The ion selective membranes of the invention can be manufactured by known processes comprising steps of dissolving the above mentioned components in an appropriate solvent, coating the solution on an electrolyte layer and drying the layer. Aging at 35°C for 24 hours is preferably performed for increasing stability of the electrode.

The ion selective electrode of the invention is not limited to the structures shown in Figs. 1 and 2 and can take any known structure. The ion selective electrode of the invention can be manufactured in the conventional manner using known materials. The conventional structures, methods and materials for manufacturing a variety of ion selective electrodes are described, for instance, in Japanese Patent Publication No. 58-4981, Japanese Patent Provisional Publications No. 52-142584, No. 57-17852, and No. 58-211648, Japanese Patent Publication No. H4-50530, United States Patents No. 4,053,381, No. 4,171,246, and No. 4,214,968; and Research Disclosure No. 16113 (September, 1977).

The determination of calcium ion in a sample blood can be performed using the composite ion selective elec-

trode of the invention as follows.

A sample blood and a reference liquid are spotted onto openings 24a and 24b of the composite ion selective electrode shown in Fig. 2, respectively. The sample
5 blood and the reference liquid reach the distributing members 26a and 26b as shown by an arrow and are supplied to the surfaces of ion selective electrode membranes of each ion selective electrode member 21a, 21b and 21c. Potential difference generated between each ion selective
10 electrode member is measured by a potentiometer 29 through the electrically connecting region at both edges of each electrode member.

The determination of standardized calcium ion at pH 7.4 can be made by measuring a calcium ion concentration and a hydrogen ion concentration from the obtained value
15 and working curve prepared in advance and then incorporating the measured calcium ion concentration and the measured hydrogen ion concentration into the following equation:

20 [Formula 1]

$$\text{Log (iCa at 7.4) = } \text{Log (iCa at pH) - 0.22 x (7.4 - pH)}$$

in which iCa means a calcium ion concentration and pH means a hydrogen ion concentration.

25 Accordingly, a calcium ion concentration and a hydrogen ion concentration can be obtained by one measurement. Further, the standardized calcium ion concentration at pH 7.4 usually required for clinical examinations can be obtained accurately and easily.

30 The composite ion selective electrode of the invention can be very favorably employed for the determination of the concentrations of calcium ion and hydrogen ion of 0.5 to 2.5 mM. However, the electrode can be also favorably employable for the determination of concentrations
35 of calcium ion and hydrogen ion of a higher level and a lower level (for example, food and other body fluids than

blood) by changing concentrations of reference liquids. Further, in case that a sample liquid is rainwater or city water, it is possible to obtain hardness, acidity and basicity thereof.

5 [Examples]

[Example 1] Calcium Ion Selective Electrode

On a polyethylene terephthalate film (support, thickness: 188 μm , sizes: 30 mm x 100 mm) was vacuum deposited a metal silver layer (thickness: approx. 800
10 nm). Both edges of the silver metal deposited layer was covered with a liquid polymer resist disclosed in Japanese Patent Provisional Publication No. 58-102146, and the center portion of the silver metal deposited layer was removed using a cutter to provide an U-shaped insu-
15 lating space.

The silver metal layer in the exposed area was brought into contact with an aqueous solution containing hydrochloric acid and a potassium hydrogen carbonate (36 mM/L of hydrochloric acid and 16 mM/L of potassium hydro-
20 gen carbonate) for approximately 60 seconds for catalytic oxidation-chlorination. Subsequently, the silver metal layer was washed with water and dried, to give a silver/silver chloride-coated electrode (laminated structure comprising a support, silver metal layer and silver chlo-
25 ride layer).

In purified water (240 g) were dissolved 28.8 g of sodium chloride and 192 g of ethanol, to prepare an electrolyte solution. The electrolyte solution was coated on the silver/silver chloride layer and dried under atmo-
30 spheric conditions. The dried layer (electrolyte layer) had an amount of 2.1 g/m².

On the electrolyte layer was formed a calcium ion selective membrane of 26 μm thick using the following composition. The prepared calcium ion selective elec-
35 trode was aged at 35°C for 24 hours.

[Composition of calcium ion selective membrane]

	Calcium di[4-(1,1,1,3-tetramethylbutyl)phenyl]	
	phosphate (t-HDOPP)	0.3 g
	Diethylphenyl phosphonate (DOPP)	3.0 g
5	Vinyl chloride-vinyl acetate copolymer	
	(VYNS)	3.0 g
	Methyl ethyl ketone	20 g

On the obtained ion selective membrane, a liquid-receiving mask made of plastic film having two liquid-receiving openings was provided, and the two openings
10 were bridged by a bridge member made of polyester yarn. Thus, a plurality of calcium ion selective electrodes of the invention were manufactured (see Fig. 1).

[Example 2]

The procedures of Example 1 were repeated except for
15 changing the thickness of the ion selective membrane to 15 μm to manufacture a plurality of calcium ion selective electrodes of the invention.

[Comparison Example 1]

The procedures of Example 1 were repeated except for
20 changing the thickness of the ion selective membrane to 34 μm to manufacture a plurality of calcium ion selective electrodes for comparison.

[Evaluation of Calcium Ion Selective Electrode]

The obtained plurality of calcium ion selective
25 electrodes were evaluated using the sample liquid and reference liquid set forth in Table 1. The evaluation procedure was performed by spotting the sample liquid and reference liquid on the openings of the ion selective assembly and measuring a standard deviation (SD) of the
30 electric potential at 60 seconds after the spotting, by means of a potentiometer (FDC 800, available from Fuji Photo Film Co., Ltd.). The results are set forth in Figures 3 to 5 and Table 1.

Table 1

5	Sample Liquid			Reference Liquid
	L	M	H	
	MOPS	40 mM	40 mM	40 mM
	NaCl	160 mM	160 mM	160 mM
10	PVP K-15	3 %	3 %	3%
	Glycerol	2 %	2%	2%
	CaCl ₂	0.5 mM	1.25 mM	1.25 mM
	pH	7.8	7.4	7.4

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Remarks: MOPS: 3-morpholinopropanesulfonic acid
PVP K-15: polyvinylpyrrolidone K-15

Each of Figures 3 to 5 graphically indicates a relationship between a period of time and an electric potential observed in the calcium ion selective electrodes of Example 1, Example 2 and Comparison Example 1, respectively.

20

Table 2

25		Thickness (μ m)	SD (mV)		
			L	M	H
30	Example 1	26	0.5	0.5	0.9
	Example 2	15	0.3	0.3	0.3
35	Com. Ex. 1	34	0.4	0.8	1.3

As is apparent from the data set forth in Figs. 3 to 5 and Table 2, the calcium ion selective electrodes according to the invention (Examples 1 and 2) gave a smaller standard deviation which meant that the variation of analytical results was smaller compared with the ion se-

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lective electrode for comparison (Comparison Example 1). Particularly, the calcium ion selective electrode having an ion selective membrane of 15 μ m thick (Example 1) showed favorable stability and potential deviation.

5 [Example 3] Hydrogen Ion Selective Electrode

On a polyethylene terephthalate film (support, thickness: 188 μ m, sizes: 30 mm x 100 mm) was vacuum deposited a metal silver layer (thickness: approx. 800 nm). Both edges of the silver metal deposited layer were
10 covered with a liquid polymer resist disclosed in Japanese Patent Provisional Publication No. 58-102146, and the center portion of the silver metal deposited layer was removed using a cutter to provide an U-shaped insulating space.

15 The silver metal layer in the exposed area was brought into contact with an aqueous solution containing hydrochloric acid and a potassium hydrogen carbonate (36 mM/L of hydrochloric acid and 16 mM/L of potassium hydrogen carbonate) for approximately 60 seconds for catalytic
20 oxidation-chlorination. Subsequently, the silver metal layer was washed with water and dried, to give a silver/silver chloride-coated electrode (laminated structure comprising a support, a silver metal layer and silver chloride layer).

25 In purified water (240 g) were dissolved 28.8 g of sodium chloride and 192 g of ethanol, to prepare an electrolyte solution. The electrolyte solution was coated on the silver/silver chloride layer and dried under atmospheric conditions. The dried layer (electrolyte layer)
30 had an amount of 2.1 g/m².

On the electrolyte layer was formed a hydrogen ion selective membrane of 20 μ m thick by a conventional method using the following composition to prepare a hydrogen ion selective electrode. The membrane was aged at 35°C
35 for 24 hours.

[Composition of hydrogen ion selective membrane]

	Tri-n-dodecylamine (TDDA)	0.1 g
	Trisethylhexyl trimellitate (Tris EHT)	3.0 g
	Potassium tetrakis(p-chlorophenyl borate) (KTpCPB)	0.06 g
5	Vinyl chloride-vinyl acetate copolymer (VYNS)	3.0 g
	Methyl ethyl ketone	20 g

On the obtained hydrogen ion selective membrane, a
10 liquid-receiving mask made of plastic film having two
liquid-receiving openings was provided, and the two open-
ings were bridged by a bridge member made of polyester
yarn. Thus, a plurality of hydrogen ion selective elec-
trodes of the invention were manufactured (see Fig. 1).

15 [Comparison Example 2]

The procedures of Example 1 were repeated except for
replacing Tris EHT (plasticizer of the ion selective
membrane) with dioctyl sebacate (DOS) to manufacture a
plurality of hydrogen ion selective electrodes for com-
20 parison.

[Comparison Example 3]

The procedures of Example 1 were repeated except for
replacing Tris EHT (plasticizer of the ion selective
membrane) with dibutyl sebacate (DBS) to manufacture a
25 plurality of hydrogen ion selective electrodes for com-
parison.

[Comparison Example 4]

The procedures of Example 1 were repeated except for
replacing Tris EHT (plasticizer of the ion selective
30 membrane) with nitrophenyl octyl ether (NPOE) to manufac-
ture a plurality of hydrogen ion selective electrodes for
comparison.

[Evaluation of Hydrogen Ion Selective Electrode]

A plurality of the obtained hydrogen ion selective
35 electrodes were stored at 45°C for 3 weeks and then evalu-
ated using the sample liquid and reference liquid set

forth in Table 1. The evaluation procedure was performed by measuring a standard deviation (SD) of the electric potential at 60 seconds after the spotting, by means of a potentiometer (FDC 800, available from Fuji Photo Film Co., Ltd.). The measurement was repeated 10 times. The results are set forth in Tables 3 and 4.

Table 3

	Plasticizer	SD (mV)		
		L	M	H
Example 3	TrisEHT	1.45	1.48	1.38
Com. Ex. 2	DOS	4.47	5.35	4.46
Com. Ex. 3	DBS	6.56	8.15	8.26
Com. Ex. 4	NPOE	12.13	6.52	10.32

Table 4

	Plasticizer	Average of Potential (mV)		
		L	M	H
Example 3	TrisEHT	- 21.70	- 1.86	21.99
Com. Ex. 2	DOS	- 7.80	9.68	30.15
Com. Ex. 3	DBS	- 1.59	3.86	4.77
Com. Ex. 4	NPOE	- 9.92	6.72	28.25

As is apparent from the results set forth in Table 3, the hydrogen ion selective electrode according to the invention (Example 3) gave a smaller standard deviation which meant that the variation of analytical results was smaller compared with the conventional ion selective

electrodes (Comparison Examples 2 to 4). Since the average of potential of the sample liquid at the M concentration level is the same as that of the reference liquid, the average of potential should show 0 mV at the M concentration level. As shown in Table 4, the average of potential observed in the hydrogen ion selective electrode of the invention is near to 0 mV at the M concentration level, which is appropriate. The averages of potential observed at the L and H concentration levels are far from the average observed at the M concentration level.

[Example 4] Composite Ion Selective Electrode

The calcium ion selective electrode prepared in Example 2, the hydrogen ion selective electrode prepared in Example 3 and a dummy electrode consisting of a support and a silver metal layer were placed between the liquid-receiving mask made of plastic film having two liquid-receiving openings and the distributing mask made of plastic film in such a manner that each support was upward and each ion selective membrane was downward. A bridge made of polyester yarn was provided for bridging the liquid-receiving openings. Thus, the composite ion selective electrode of the present invention was prepared (see Fig. 2).

[Effect of Invention]

The present invention can provide a calcium ion selective electrode which shows good analytical reproducibility and which gives a constant analytical data within a short period of time by making the thickness of a calcium ion selective membrane smaller than a certain thickness. Particularly, a calcium ion selective electrode having satisfactory reproducibility and ability as electrode can be manufactured by employing a thin ion selective membrane made of a specific material.

Further, the present invention can provide a hydrogen ion selective electrode which is almost free from

deterioration of ability as electrode after lapse of time so as to show remarkably high reproducibility of analytical data after lapse of time by employing a hydrogen ion selective membrane made of a specific material.

5 Accordingly, the composite ion selective electrode of the invention shows satisfactory reproducibility of analytical data and ability as electrode. Determination of concentration of a calcium ion in blood can be performed with high accuracy by employing the composite ion
10 selective electrode of the invention which shows high ability and reproducibility of analytical data.

[Brief Description of Drawings]

[Fig. 1]

15 Fig. 1 is a rough perspective view of a structure of an ion selective electrode for determining a calcium ion or a hydrogen ion.

[Fig. 2]

Fig. 2 is a broken perspective view of a structure of a composite ion selective electrode of the invention.

20 [Fig. 3]

Fig. 3 is a graph indicating a relationship between a period of time and an electric potential observed in the use of an ion selective electrode of Example 1.

[Fig. 4]

25 Fig. 4 is a graph indicating a relationship between a period of time and an electric potential observed in the use of an ion selective electrode of Example 2.

[Fig. 5]

30 Fig. 5 is a graph indicating a relationship between a period of time and an electric potential observed in the use of an ion selective electrode of Comparison Example 1.

[Description of Symbols]

11: an electro-insulating support
35 12a, 12b: a silver layer
13a, 13b: a silver chloride layer

- 14: an electrolyte layer
- 15: an ion selective membrane
- 16: an electro-insulating member
- 17a, 17b, 24a, 24b: an opening for introducing liquid
- 5 18, 25: a bridge member
- 21a, 21b, 21c: an ion selective electrode member
- 22: an upper frame
- 23: a lower frame
- 26a, 26b: a distributing member

[Title of Document] Sheet for Summary

[Summary]

[Object] The object is to provide an ion selective electrode for determining a concentration of a calcium ion
5 and a hydrogen ion with high accuracy and reproducibility of analytical data.

[Means for Solving] A composite ion selective electrode comprising a calcium ion selective electrode member, a hydrogen ion selective electrode member, an electro-insu-
10 lating member having two openings for introducing liquids, a pair of distributing members for distributing each liquid to each ion selective electrode member, and a bridge member to electrically connect the introduced liquids, wherein the calcium ion selective membrane of said
15 calcium ion selective electrode member has a thickness of 5 to 30 μm , and the hydrogen ion selective membrane of said hydrogen ion selective electrode member contains tri-n-dodecylamine and trisethylhexyl trimellitate; each
20 ion selective electrode member and a method for determining the concentration of a calcium ion.

[Selected Drawings] Figure 2

FIG. 1

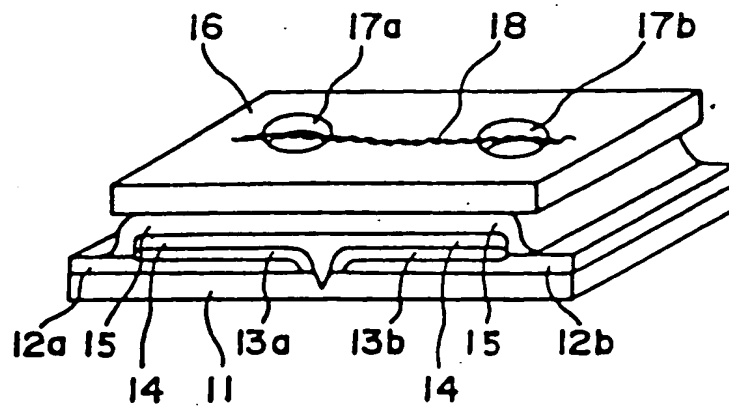


FIG. 2

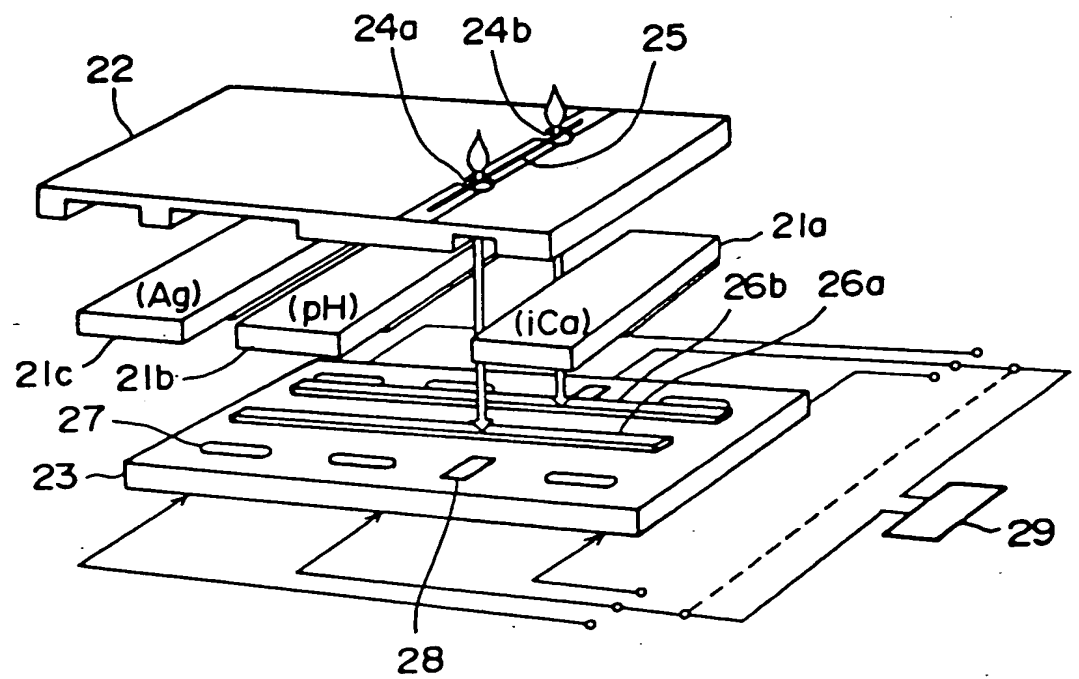


FIG. 3

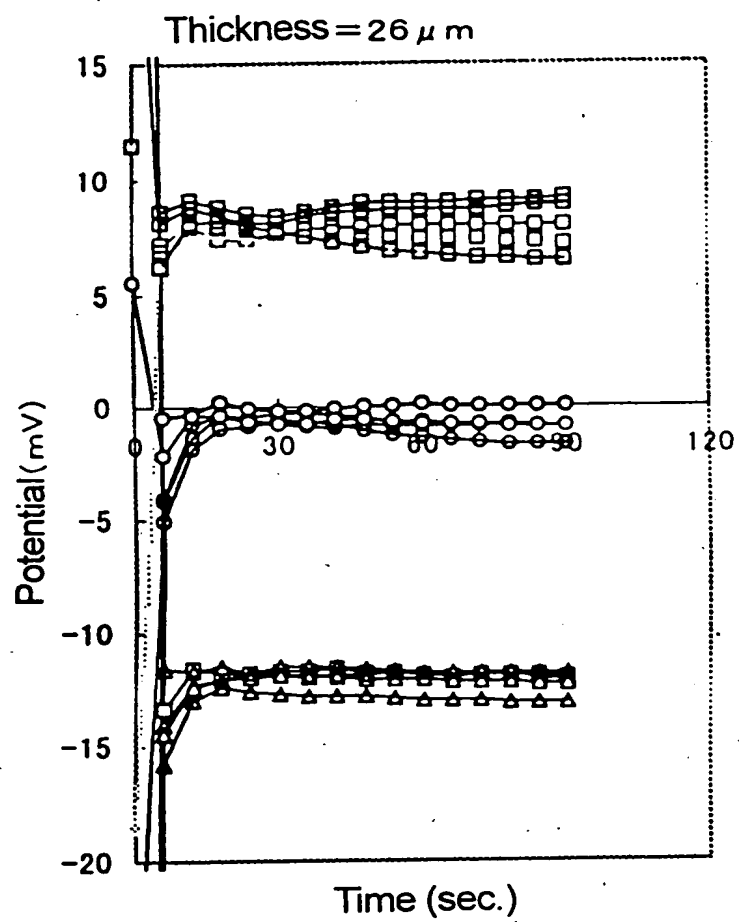


FIG. 4

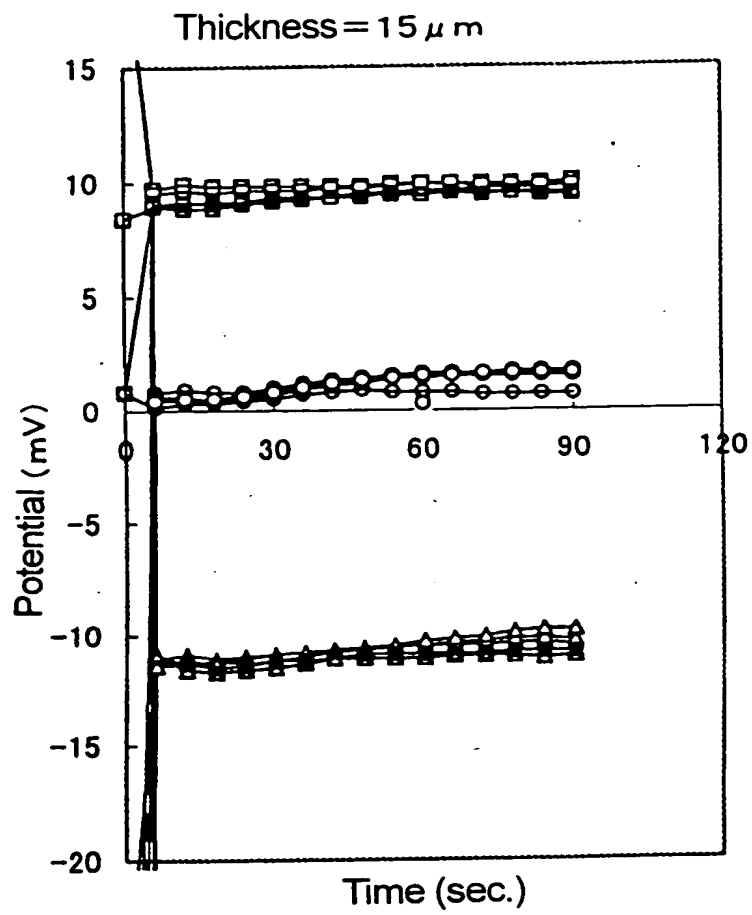


FIG. 5

